Remarks

Applicant and the undersigned would like to thank the Examiner for her efforts in the examination of this application. Reconsideration is respectfully requested.

I. Restriction Pursuant to 35 USC 121

The Examiner has imposed a restriction requirement between Group I, Claims 1-13, drawn to a method for measuring an evolution rate, and Group II, Claims 14-25, drawn to a device for measuring an evolution rate.

Applicants have elected to pursue Group I, Claims 1-13, at the present time, and consequently Claims 14-25 have been withdrawn.

II. Objection to the Specification

The Examiner has objected to the Specification for nonenablement.

To address these objections, the inventors have provided the following explanations:

1. The microrespirometer is used to determine the rate of CO_2 production of a sample of solid, liquid, or a mixture of the two under shaking conditions. Many liquid samples, such as water, wastewater, and milk require shaking to get a reproducible CO_2 production rate.

2. The rotary shaking of the microrespirometer method is for the purpose of homogenizing the alkaline solution during the process of absorbing CO_2 . A constant shaking rate is required, in the range of 150-300 rpm as experienced in the experiments.

3. The equilibrium of the microrespirometer method is referring to the equilibrium between the CO_2 production (evolution) from a sample and the CO_2 absorption of the alkaline-indicator solution. When the CO_2 production rate of a sample and the CO_2 absorption rate of the alkaline-indicator solution become equal, the headspace CO_2 concentration becomes constant. Since the CO_2 absorption rate of the alkaline-indicator solution is proportional to the headspace CO_2 concentration, equilibrium between the CO_2 production and absorption in the system would eventually be achieved when the CO_2 production rate equals absorption rate. For example, the CO_2 absorption rate of a 1 mM alkaline-indicator solution was around 62 μL/hr, under the conditions specified in the experiment when the headspace CO_2 concentration was 400 ppm (the usual ambient CO_2 in the laboratory). If the CO_2 production rate of a sample is 10 μL/hr, the headspace CO_2 concentration would go down because the absorption rate is greater than the production rate. As the headspace CO_2 concentration goes down, so does the CO_2 absorption rate of the alkaline-indicator solution. The headspace CO_2 concentration eventually would reach a constant level around 40 ppm, when the CO_2 absorption rate of the alkaline-indicator solution is precisely the same as the CO_2 production rate (10 μL/hr). The CO_2 absorption rate of the alkaline solution is then determined at this equilibrium, which is precisely the CO_2 production rate of the sample. If the CO_2 production rate of a sample is greater than the CO_2 absorption rate of the alkaline solution, the headspace CO_2 concentration will go up. As the headspace CO_2 concentration goes up, so does the CO_2 absorption rate of the alkaline solution until equilibrium is reached at a higher CO_2 concentration where the CO_2 production rate equals the CO_2 absorption rate. The actual CO_2 concentration of the headspace does not need to be known in order to know the CO_2

production rate of the sample, but only the CO_2 absorption rate of the alkaline-indicator solution at the equilibrium headspace concentration. This is one of the unique features of the invention, since all other known methods need to know the CO_2 concentration before and after incubation in order to calculate the CO_2 production rate by the difference. The microrespirometer method thus eliminates the complications associate with the determination of CO_2 concentration of the headspace. This improved approach not only differs from the prior art in principle but also achieves higher precision and sensitivity with a shorter assay time.

The caption of FIG. 6 has been corrected as suggested by the Examiner, and the citation to FIG. 6 has been added on page 12, line 11, which details the samples used.

III. Rejection of Claims 1-13 under 35 USC 112

The Examiner has rejected Claims 1-13 under 35 USC 112, first and second paragraphs.

The preamble of Claim 1 has been amended to recite "A method for measuring an evolution rate of ~~a gas~~ <u>carbon dioxide</u> from a sample".

The first step of Claim 1 has also been amended to recite "<u>placing</u> a sample <u>in gas communication</u> with a solution . . ."

Claims 2 and 3 have been commensurately amended to replace "equilibrating" with "<u>placing</u>". The Examiner's rejection here is not understood, as there is only one evolution rate recited, and that is in Claim 1.

Claim 8 has been amended to recited that substantially all of the solution is withdrawn.

Claim 13 has been amended to recite that *M* is the <u>alkaline</u> concentration of the solution, as the Examiner has suggested.

IV. <u>Rejection of Claims 1-13 under 35 USC 103(a)</u>

The Examiner has rejected Claims 1-13 under 35 USC 103(a) as being unpatentable over Baker et al. (Abstract, 1999) in view of Harp (US 6,368,870).

This rejection is respectfully traversed. The microrespirometer method of the present invention is not a method for CO_2 concentration determination but for CO_2 production rate determination. One of the novel features of the invention is the ability to determine the CO_2 production rate of a sample without the complication of determining CO_2 concentration. The methods disclosed in the prior art require a knowledge of temperature, pressure, and headspace volume to e CO_2 concentration before and after incubation in order to calculate the CO_2 production rate. While temperature may be easily determined, pressure and headspace volume are difficult to determine, as solid samples usually do not have a regular shape and the pressure in a closed system can vary due to vapor production in the system. In addition, a gas standard of known CO_2 concentration must be included for CO_2 concentration calibration, which is not conveniently available. The present invention bypasses all the complications associated with the determination of CO_2 concentration and instead directly assesses the CO_2 production rate of a sample in solid, liquid, or a mixture of both forms.

The methods of Baker, Harp, and Rowell as cited by the Examiner are all directed to CO_2 concentration determinations. The equilibrium vapor method of Baker addresses the equilibration of a water-air system that has constant inorganic carbon content. Baker's

method is not suitable for samples that constantly produce CO_2 (respiration) because no equilibrium can be reached with such a variable inorganic carbon system. In contrast, the microrespirometer method of the present invention applies to a system in which CO_2 may constantly be produced from a sample. The equilibrium of the microrespirometer method refers to an equilibrium between the CO_2 production from a sample and the CO_2 absorption by the alkaline-indicator solution (see point 3 above under §II). The term "equilibrium" referred in the present application thus is totally different from that referred in Baker's method.

The microrespirometer method is fundamentally different from the method of Rowell. Rowell's method is for respiration measurement; however, it is based on the determination of headspace CO_2 concentration before and after an incubation period. Rowell subsamples the headspace air and equilibrates it with a bicarbonate-indicator solution in a closed Vacutainer tube and uses the equilibrium vapor method (same as Baker's) to assess the CO_2 concentration, that is, equilibrating the headspace CO_2 with a solution (or water) that has constant total inorganic carbon content. None of the previous methods can determine the CO_2 production rate of a sample without first determining CO_2 concentration. The microrespirometer method of the present invention is currently the only method that determines the CO_2 production rate of a sample without the need to know CO_2 concentration. Thus the present method bypasses the complication and tediousness of determining CO_2 concentration, temperature, pressure, and headspace volume.

Further, the controlled diffusion analysis method taught by Harp is not analogous to the present invention, and cannot be used to determine the respiration rate of a sample.

Therefore, it is respectfully believed that the present Claims 1-13 patentably define over the cited art.

Conclusions

Applicants respectfully submit that the above amendments place this application in a condition for allowance, and passage to issue is respectfully solicited. The Applicants and the undersigned would like to again thank the Examiner for her efforts in the examination of this application and for reconsideration of the claims as amended in light of the arguments presented. If the further prosecution of the application can be facilitated through telephone interview between the Examiner and the undersigned, the Examiner is requested to telephone the undersigned at the Examiner's convenience.

Respectfully submitted,

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CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that the foregoing is being sent by facsimile transmission to Examiner Yelena G. Gakh, Art Unit 1743, U.S. Patent and Trademark Office, facsimile number 1-703-872-9306, this 22nd day of October, 2004.

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